Love is in the air: sociality and pair bondedness influence sifaka reproductive signalling

Lydia K. Greene[a], Christine M. Drea[a,b,*]

[a] Department of Evolutionary Anthropology, Duke University, Durham, North Carolina, United States of America; [b] Department of Biology, Duke University, Durham, North Carolina, United States of America

* Correspondence: C.M. Drea, Department of Evolutionary Anthropology, Duke University, 129 Biological Sciences Bldg., Science Drive, Box 90383, Durham, NC 27708-0383, USA. *E-mail address*: cdrea@duke.edu (C.M. Drea)

Abstract

Social complexity, often estimated by group size, is seen as driving the complexity of vocal signals, but its relation to olfactory signals, that arguably arose to function in nonsocial realms, remains underappreciated. That olfactory signals also may mediate within-group interaction, may vary with social complexity, and may promote social cohesion underscores a potentially crucial link with sociality. To examine that link, we integrated chemical and behavioural analyses to ask if olfactory signals facilitated reproductive coordination in a strepsirrhine primate, the Coquerel's sifaka (*Propithecus coquereli*). Belonging to a clade comprising primarily solitary, nocturnal species, the diurnal, group-living sifaka represents an interesting test case. Convergent with diurnal, group-living lemurids, sifakas expressed chemically rich scent signals, consistent with the social complexity hypothesis for communication. These signals minimally encoded the sex of the signaler and varied with female reproductive state. Likewise, sex and female fertility were reflected in within-group scent investigation, scent marking, and over-marking. We further asked if, within breeding pairs, the stability or quality of the pair's bond influenced the composition of glandular signals and patterns of investigatory or scent-marking behaviour. Indeed, reproductively successful pairs tended to show greater similarity in their scent signals than did reproductively unsuccessful pairs, potentially through chemical convergence. Moreover, scent marking was temporally coordinated within breeding pairs and was influenced by past reproductive success. That olfactory signalling reflects social bondedness or reproductive history lends support to recent suggestions that the quality of relationships may be a more valuable proxy than group size for estimating social complexity. We suggest that olfactory signalling in sifakas is more complex than previously recognized and, as in other socially integrated species,

can be a crucial mechanism for promoting group cohesion and maintaining social bonds. Thus, the evolution of sociality may well be reflected in the complexity of olfactory signalling.

Keywords: chemical communication; GC/MS; glandular secretion; group cohesion; olfactory signal; primate; reproduction; scent marking; sifaka; social complexity.

The social complexity that characterizes individualized animal societies has been shown to influence behaviour in various and profound ways (de Waal & Tyack 2003). For example, the 'social brain hypothesis' posits that intelligence, particularly in primates, arose to handle the computational demands of social living (Byrne & Whiten 1988; Dunbar 1998). Likewise, the 'social complexity hypothesis' posits that group living influenced the evolution of communication systems, specifically selecting for individually distinctive (Tibbetts 2004; Pollard & Blumstein 2011) or increasingly complex vocal and visual signals (reviewed in Freeberg et al. 2012). Recently, despite an historical focus on asocial species in studies of olfactory communication, aspects of social organization also have been linked to scent-marking behaviour (Becker et al. 2012) and the complexity of olfactory signals (delBarco-Trillo et al. 2012). Nevertheless, defining a representative measure of social complexity remains elusive. Group size has served as its proxy in studies of social intelligence (Dunbar 1993, 1998) and vocal communication (McComb & Semple 2005; Freeberg 2006), but arguably fails to capture the unique nature of intricate relationships (see Cheney et al. 1986; Barrett & Henzi 2005; Maestripieri 2005; Silk 2007; Freeberg & Lucas 2012), particularly in small or family groups. Some researchers have therefore deemphasized group size as a metric of social complexity in favour of the stability of social bonds (Dunbar & Schultz 2007). To the extent that bonded relationships may have influenced the evolution of encephalization across vertebrates (Dunbar & Schultz 2007; Schultz et al. 2011), so too may they have influenced the complexity of communicatory elements. Here, we explore the latter relationship in a primate and ask if, within species, variation in pairwise bondedness reflects the richness of olfactory communication.

Scent marking is often inextricably linked with territorial or reproductive advertisement (Ralls 1971; Eisenberg & Kleiman 1972; Johnson 1973), particularly to broadcast the signals of

asocial species (Alberts 1992), but also can be a potent mechanism for mediating more immediate *intragroup* interaction (reviewed in Scordato & Drea 2007). For socially integrated species that also rely on scent marking, the social complexity hypothesis of communication predicts increased signal richness. In strepsirrhine primates (including lorises, galagos, and lemurs: Schilling 1979), this pattern of increasing signal complexity with increasing social complexity appears to hold. The mode of deposition (Schilling 1980) and the degree of chemical richness (delBarco-Trillo et al. 2011) of olfactory signals broadly reflects variation in phylogeny and socioecology. Notably, the nocturnal and relatively solitary species (in Galagidae, Lorisidae, Daubentoniidae, and Cheirogaleidae) rely primarily on urinary cues that contain fairly simple chemical blends and purportedly simple messages (delBarco-Trillo et al. 2011). By contrast, the social and diurnal species (in Lemuridae) rely primarily on a suite of specialized glandular signals that are chemically diverse and encode a wide range of signaller information (Scordato et al. 2007; Charpentier et al. 2008; Boulet et al. 2009, 2010) that is salient to receivers (Harrington 1979; Palagi et al. 2004; Scordato & Drea 2007; Charpentier et al. 2010), including group members. Comparative chemical analyses of glandular secretions even reveal a relationship between odorant richness and the type of social or dominance system (delBarco-Trillo et al. 2012). Thus, the evolution of scent-signal complexity in strepsirrhines (delBarco-Trillo et al. 2011) mirrors the co-evolutionary change in primates from nocturnality to diurnality and from solitary to social living (see Schultz et al. 2011).

The fact that olfactory behaviour can be socially facilitated (Glickman et al. 1997; Jordan et al. 2007) or that olfactory cues can be coupled with visual and auditory features to draw immediate attention (Bekoff 1979; Partan & Marler 2005; Drea & Scordato 2008; Clarke et al. 2009) further highlights the intragroup relevance of scent signalling. Whether based on the

information contained in the chemical matrix of a scent, on the frequency and patterning of scent deposition, or on the behavioural responses following odorant discovery, olfactory communication in socially integrated species often serves to enhance group cohesion. Social cohesion may be promoted, for instance, via group-specific, chemical signatures (Bloss et al. 2002; Theis et al. 2012), acquired by allo-marking (Buesching et al. 2003) or gradual scent convergence (Safi & Kerth 2003) and potentially implicating shared microbial communities (Albone 1984; Archie & Theis 2011). Likewise, scent marking facilitates the establishment or maintenance of social bonds (Butler & Butler 1979; Kleiman 1981; Mertl-Millhollen et al. 1986; Overdorff & Tecot 2007), with animals habitually increasing costly scent deposition specifically during the period of bond formation (Rothman & Mech 1979; Porton 1983; Savage et al. 1988; Kranz 1991). Additionally, in social carnivores, group hunting may be coordinated, in part, by the scent trails of leaders, allowing laggards to track pack movements (Estes & Goddard 1967). Sniffing between group members, particularly in fission-fusion societies, can be an important means of reaffirming relationships (Kruuk 1972). Even the social context of sniffing can influence cohesion or organize group action (Glickman et al. 1997). We refer to this functional relationship – the specifically social benefit, in this case, of olfactory signalling – as the 'social cohesion hypothesis' of communication.

Here, we combine chemical and behavioural approaches to probe the complexity and social function of olfactory communication in the Coquerel's sifaka (*Propithecus coquereli*). Sifakas present an interesting test case, both for examining scent-signal complexity within a phylogenetic framework and for examining the social cohesion hypothesis.

With regard to scent-signal complexity, if the elaboration of signalling has been driven by a shift towards diurnality and sociality, as appears to be the case in Lemuridae, we would expect

to see convergent evolution (i.e., comparable complexity) in the signalling of sifakas. Consistent with their primarily nocturnal and solitary relatives (see Horvath et al. 2008), sifakas have maintained olfactory reliance on urine (Mertl-Millhollen 1979), but they produce relatively richer urinary scent signals (delBarco-Trillo et al. 2011; see electronic Supplementary Material, Fig. S2). In accordance with their diurnal and social lifestyle (Richard et al. 1993, Erkert & Kappeler 2004), sifakas additionally rely on glandular secretions (Schilling 1979; Supplementary Fig. S1) that carry chemical information about the signaller (Morelli et al. 2013). Although there is currently little evidence that seasonal variation in scent-marking behaviour reflects the signaller's reproductive state (Brockman 1999; Lewis 2005; Pochron et al. 2005), there is broad temporal coordination between the sexes (Brockman 1999), with marks possibly conveying dominance status (Lewis 2005) and sex (female bias: Brockman 1999; male bias: Lewis 2005; Pochron et al. 2005).

With regard to the social cohesion hypothesis, we expect a macrosmatic species living in an individualized society (see de Waal & Tyack 2003) to rely on olfactory signalling in intragroup coordination. Specifically, we expect chemical convergence in scent-signal composition, as well as behavioural coordination in scent-signal deposition, between established mates. Sifakas live in mixed-sex, female-dominant and female-philopatric groups of 2-14 individuals (Kubzdela et al. 1992; Richard et al. 1993; 2002). Over a third of groups contain only one unrelated, adult male and he maintains a relatively long tenure (Kappeler & Schäffler 2008). Despite female promiscuity (Brockman 1999), the dominant male sires over 90% of the offspring (Kappeler & Schäffler 2008), with reproductive skew being maintained by behavioural and hormonal suppression of subordinate males (Kraus et al. 1999; Lewis 2008). Thus, adult female sifakas and their mates establish long-term, stable social bonds, and potentially cohabitate and/or

reproduce together for years – factors that reflect this species' social complexity in a manner not captured by group size. We thus differentiate the breeding pairs in our study by two potentially independent metrics of bondedness -- their familiarity with one another (i.e., the duration of their cohabitation) and their past reproductive history (i.e., whether they had successfully reproduced or not).

In the chemical portion of our study, we use gas chromatography and mass spectrometry (GC/MS) to determine the volatile composition of odorants collected from the genital glands of both sexes across different phases of the reproductive season (i.e., pre-breeding, breeding, and post-breeding). Consistent with the social complexity hypothesis, we expect the chemical composition of sifaka genital secretions to contain information comparable in richness to that detected in Lemuridae. Minimally encoded could be signaler sex and fertility, pertinent to reproductive coordination. Consistent with the social cohesion hypothesis, we predict that, via any functional means of chemical convergence, strongly bonded pairs (i.e., long-established and/or reproductively successful pairs) will express more similar chemical profiles than will weakly bonded pairs (i.e., newly established and/or reproductively unsuccessful pairs).

In the behavioral portion of our study, we observe potential mates across the same three phases of the reproductive season to identify various social and sexual factors that might influence genital scent marking in sifakas. We expect that these factors could act in tandem. If, as in Lemuridae, sifaka olfactory behaviour functions to facilitate reproduction, we would expect the sexes to increase scent marking and scent investigation during periods of advertisement, notably in the pre-breeding and breeding seasons. If their olfactory behaviour additionally serves to establish and maintain social bonds, we would expect scent investigation or marking by each member of a breeding pair to reflect the current state of their bond. We therefore expect temporal

158 coordination between pair members, such that one animal's scent-marking frequencies, for
159 example, would mirror those of its partner. Because scent deposition is costly (Gosling et al.
160 2000; Roberts et al. 2001), animals should be economical and engage in this behaviour only
161 when the benefits outweigh the costs. We thus anticipate the frequency of scent deposition by
162 pair members to be influenced by their bondedness, such that weakly bonded pairs would invest
163 more heavily in olfactory signalling than would strongly bonded pairs.
164
165 **Materials & Methods**
166 *Subjects and Housing*
167 Our study population derived from six groups of sifakas, totaling 25 animals, all of which
168 were housed at the Duke Lemur Center (DLC) in Durham, North Carolina. Each group
169 comprised a dominant, reproductively intact pair of potential mates (hereafter 'breeding pair')
170 that had cohabited for varying durations, as well as various relatives or offspring of at least one
171 of the dominant animals (Table 1). The breeding pairs, plus an additional three subordinate, adult
172 subjects (to maximize sample size), served as focal subjects in our one-year chemical study
173 ($n_{chemistry} = 15$). Only the breeding pairs served as focal subjects in our two-year behavioural
174 study ($n_{behaviour} = 12$).
175 As Coquerel's sifakas are endangered (Andrainarivo et al. 2008), breeding pairs are
176 formed through DLC husbandry practices, following Species Survival Plan recommendations to
177 maximize genetic diversity of future offspring. Thus, adult animals are paired based on
178 minimizing relatedness – a process that has been successful in creating social groups that, in age
179 and sex composition, mirror sifaka groups seen in the wild (Richard et al. 1993, 2002; Kappeler
180 & Schäffler 2008). Although pairs may differ in their reproductive success, to date, none of the

paired animals in our study have been separated for social reasons. The reproductive success of these breeding pairs prior to and during the study is provided in Table 1.

Each of the six groups occupied a separate, large (1575 ft^3/individual) indoor/outdoor pen (hereafter referred to as the 'pen' housing condition) year round. In both study years, three of the groups additionally had access to large (1.48-6.0 acres) forested enclosures (hereafter referred to as the 'forest' housing condition), where they could semi-free range from mid-May to mid-November or when ambient temperatures stayed above 5 °C. In the second year of the study, a fourth group also gained forest access. Thus, roughly half of the subjects experienced the pen condition only, whereas the other half experienced a combination of the pen and forest conditions. Throughout the year, all of the subjects were fed a once-daily diet of Leaf-Eater Primate Diet Mini-Biscuit (#5672, Mazuri, Brentwood, MO), accompanied by fresh vegetables, beans, nuts, and freshly cut leaves from local flora. While semi-free ranging, the individuals in the forest condition additionally foraged on local vegetation. Water was always freely available.

We could easily identify each individual via colored collars, tail shaves, and distinguishing markings. All of the subjects were healthy and in good condition for the duration of the study. The animals were maintained in accordance with the US Department of Agriculture regulations and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The research protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Duke University (protocol #A171-09-06).

Study Periods

We conducted our study during two 6-month periods, encompassing two consecutive breeding seasons, in 2010 and 2011. For sifakas in the Northern Hemisphere, breeding occurs

primarily in July-September and births occur primarily in December-February (DLC unpublished records). Using DLC life-history records, we selected three time periods, *a priori*, to broadly represent the pre-breeding (all of June), breeding (last week of July-first week of September), and post-breeding (all of October-first week of November) seasons. The intervals we incorporated between seasons served to exclude transitional periods. In both study years, all of the observed mating or sexual behaviour occurred during the breeding season, thusly defined.

Odorant Sample Collection and Preparation

For our chemical study, we collected samples of genital odorants from each focal subject, roughly once per season (totaling n = 36 odorant samples). From the 7 adult females, we obtained 17 labial scent samples (representing 5, 6 and 6 individuals in the pre-breeding, breeding and post-breeding seasons, respectively). From the 8 adult males, we obtained 19 scrotal scent samples (representing 5, 7 and 7 individuals in the pre-breeding, breeding and post-breeding seasons, respectively). We additionally sampled male sternal secretions; however, results from the chemical analyses of sternal secretions will be presented elsewhere, as here, we focus only on the secretions produced by both sexes. For these collection procedures, trained DLC personnel captured and gently restrained the subjects while we used clean forceps to rub cotton swabs (previously washed with methanol and pentane) across the glandular fields. The samples were immediately enclosed in solvent-washed chromatography vials, placed on ice, and stored at -80 °C within 2 hours of collection, where they remained until analysis.

We extracted the volatile components of the scent samples from cotton swabs using a protocol that has been previously described (Drea et al. 2013). Briefly, while keeping samples on ice, we added 500 µL of methyl-*tert*-butyl-ether (MTBE) and 500 µL of ultrapure water to each

sample. We then vortexed the vials for 45 s, centrifuged the samples for 5 min at 3000 rpm, and removed the solvent fraction by pipetting it into a clean chromatography vial. We repeated the procedure twice more by adding an additional 500 µL of MTBE to each cotton swab. We stored extracted samples at -80 °C until further analysis. We processed all odorant samples within 6 months of collection. As shown in other primate species (Scordato et al. 2007; Lenochova et al. 2008), freezer storage does not alter the quality of odorants over this relatively short duration.

Prior to GC/MS analysis we removed a 500-µL aliquot of the MTBE extract layer of each sample and concentrated it to 50-100 µL. We then transferred the samples to a solvent-washed chromatography insert and added 5 µL of an internal standard, hexachlorobenzene (HCB, 1 mg/mL) to verify the consistency of retention times between runs. We analyzed all samples within 24 hours of concentration.

GC/MS Analysis and Compound Identification

We analyzed the odorant samples via GC/MS using protocols previously described for various strepsirrhines (Drea et al. 2013). Briefly, we injected 1 µL of sample extracts into a Shimadzu GCMS-QP2010 instrument (Shimadzu Scientific Instruments) equipped with a Shimadzu AOC-20 series autosampler, and ran samples on a Restek SHR5XLB (30 m x 0.25 mm x 0.25 µm, Shimadzu Scientific Instruments). The injector temperature was 280 ºC, the ion source temperature was 200 ºC, and we used helium as the carrier gas. We ran samples using a 43-min protocol, run in splitless mode, during which the temperature was ramped between 80 ºC and 180 ºC (20 ºC/min) after a 3-min solvent delay, and then ramped between 180 ºC and 320 ºC (5 ºC/min). The final temperature was held for 7 min.

We detected volatile chemicals using the GCMSsolution POSTRUN ANALYSIS software (v. 2.50; Shimadzu Scientific Instruments) and verified peaks manually. We standardized the retention times (*rt*) of peaks using both our internal standard, HCB (*rt* = 12.6 min), and an endogenous compound, squalene (*rt* = 28.5 min), which is a frequent component of strepsirrhine signals (Drea et al. 2013). We then manually aligned peaks using the standardized *rt* and molecular weight and, when possible, identified compounds using *rt*, molecular weight, mass spectra, and comparisons to mass spectral libraries (National Institute of Standards and Technology, Wiley Registry). We retained peaks that were present in minimally two samples, produced a regular *rt*, and whose relative abundance represented minimally 0.05% of the total chromatographic area.

Chemical Analyses

To explore the chemical information conveyed in sifaka genital secretions, we used an analytical approach combining Principle Component Analysis (PCA) with Linear Discriminant Analysis (LDA), as described by Drea and colleagues (2013). In particular, owing to the large number of compounds identified, PCA (applied to the relative abundances of these compounds) was first necessary to reduce the dimensionality of our dataset. Nevertheless, because our dataset was sparse (i.e., not all subjects expressed all compounds identified) and PCA is not particularly robust against large numbers of zeros, we included only the subset of compounds that were expressed by all individuals. This approach conservatively underestimates the differences between groups (see Drea et al. 2013).

Given that semi-free ranging animals may have foraged certain foods in the forest that were less available to animals in the pen condition, we first addressed if housing condition may

have affected the chemical composition of sifaka secretions. In 2010, we had seven animals (3 females and 4 males) in the pen condition only, and eight animals (4 females and 4 males) in the mixed pen and forest conditions. We ran a single PCA using each focal subject's mean relative abundances of chemicals (i.e., averaged across the three seasons). We retained all PCs with eigenvalues greater than 1 that explained at least 1% of the variation. We used these PCs as covariates in an LDA for which housing condition (assigned once for whether an animal always occupied a pen or sometimes also ranged in a forest) was the X category. Using a Wilks' λ Test of group differences, we found that housing condition (i.e., pen only vs. mixed pen and forest) had no effect on the chemical composition of its odorants (see electronic Supplementary Material, Fig. S3), so we discounted housing condition in our subsequent chemical analyses.

Using the same method combining PCA and LDA as described above, we then examined whether the sexes produced different chemical signatures, again entering the mean relative abundance scores (averaged across seasons) into the PCA. Lastly, to test for 'fertility' effects on chemical signals, we analyzed seasonal variation in odorant composition by running a separate PCA for each sex, this time separating the samples by season. In these analyses, we retained PCs that fulfilled the same criteria as in the previous 'housing-condition' analysis and used them as covariates in three separate LDAs for which we entered as the X category either sex, female season, or male season. We performed all PCA/LDA statistical analyses using the JMP 10 statistical software package (SAS institute, 10.0.0).

We next asked if the quality or strength of the social bond between male and female sifakas was reflected in the composition of their volatile chemicals. Our measures of bondedness included duration of cohabitation and past reproductive success. Specifically, we tested if the chemical distance between individuals covaried with these measures of bondedness. We focused

these analyses on eight mixed-sex dyads that included our six breeding pairs and two additional pairs. The latter two pairs each comprised of one of the dominant animals, plus an unrelated, subordinate adult of the opposite sex. Given the sifaka's mating system, subordinate adults living within the same social group could be considered potential mates. Because chemical distance and genetic distance are positively correlated in other strepsirrhines (Charpentier et al. 2008; Boulet et al. 2009), we excluded one subordinate male that was housed with his parents. For these analyses, we retained only the subset of chemical compounds (N = 233) that were shared by both sexes (i.e., minimally expressed by 1 male and 1 female). Using a presence/absence coding scheme in PC-ORD 5.20 (McCune & Grace 2002), we estimated the relative Euclidean distances between the chemical profiles of our dyad members (see Boulet et al. 2009). We then used a linear regression (JMP 10.0) to examine the relationship between pairwise chemical distance and the number of years a pair had cohabitated, and a Mann-Whitney U-test (Sokal & Rohlf 1995) to test pairwise chemical distance against the pair's past reproductive success. Given our prediction of chemical convergence (i.e., greater similarity) with increasing social bondedness, we performed a one-tailed test.

Behavioural Observations

We observed the olfactory behaviour of our six breeding pairs of sifakas (while they were housed in their habitual groups), during 30-min focal sessions, conducted in randomized order during the morning hours (8 am-12 pm). In each focal session, we observed both pair members concurrently and, using a Psion Workabout and the Observer software package, scored the relevant behaviour of both animals. Specifically, we recorded the frequency of sniffing and licking of deposited scent marks, as well as the frequency of genital and urine marking. Because

certain behaviour tended to co-occur, we ultimately collapsed sniffing and licking of odorants into a category of 'mark investigation,' and similarly collapsed genital and urine marking into a category of 'urogenital marking.' We additionally recorded if a scent mark was a new mark (i.e., deposited in an area devoid of olfactory activity for >1 min) or an overmark (i.e., deposited on top of a previous scent mark, within 1 min of the prior mark's deposition). Lastly, we also recorded the frequency of male-specific olfactory behaviour, including sternal marking and tree gouging; however, the findings pertaining to male-specific behaviour will be presented elsewhere, as here, we focus only on the behaviour common to both sexes. Animals were scored as being out of view if their behaviour could not be reliably assessed. We conducted these observations across the pre-breeding, breeding, and post-breeding seasons of 2010 and 2011. This regimen amounted to 610 focal sessions, totalling 305 hours of actual observation, but representing 610 animal observation hours.

Because a single observer collected the behavioural data, we assessed intra-observer reliability using video recordings of our focal subjects. We video-recorded behaviour under conditions identical to those during our observation sessions, both when subjects were housed in their pens and when they were semi-free ranging in the forest. The observer scored these videos in random order, on two separate occasions. We calculated indices of concordance for mark investigation, urogenital marking, and urogenital overmarking as the percentage agreement between the two datasets (Martin & Bateson 1993). The index of concordance for mark investigation was 97%, and indices for both urogenital marking and overmarking were above 99%. These percentages did not differ for videos recorded in the pen or forest conditions.

Behavioural Analyses

We ran three separate generalized linear mixed models (GLMMs) to test for social and seasonal effects on (1) mark investigation, (2) urogenital marking, and (3) urogenital overmarking in sifakas. Both mark investigation and urogenital marking were scored as frequencies and, therefore, were modeled initially using a Poisson distribution; however, after determining that the datasets were both overdispersed (i.e., the variance was greater than the mean) and zero-inflated, we subsequently re-ran the models using the zero-inflated negative binomial distribution (ZINB) with a log-link function (Zuur et al. 2012). For urogenital overmarking, we first calculated the proportion of overmarks (i.e., the number of marks that were deposited on top of a previously established mark/total number of marks) to standardize for differences in absolute frequency of urogenital marking. We subsequently modeled these proportional data using the binomial distribution with the logit-link function (Zuur et al. 2012).

We included the animal's concurrent housing condition (two classes: pen and forest) as an explanatory variable in the models. For this variable, we scored whether the animals that had access to semi-free ranging conditions were actually housed in their pen or in the forest at the time of observation. As the immediate housing condition did influence certain aspects of olfactory behaviour, we retained this variable in our behavioural analyses. We additionally included the following suite of explanatory variables in all models: sex (two classes: male, 'M' and female, 'F'), season (three classes: pre-breeding, breeding, and post-breeding), study year (two classes: 2010 and 2011), age (continuous variable, in years), duration of the pair's cohabitation (continuous variable, in years), and reproductive success in past years (two classes: yes and no). To account for any focal periods that deviated from 30 min in the analyses of mark investigation and urogenital marking, we included an offset of the log of the duration, in hours, of each observation period. In all models, we nested the individual's identity within the group, as

a random effect, to account for non-independence of a given individual's data points. We determined the best-fit models via stepwise deletion, removing the variable with the highest *P*-value, and re-fitting the model until only significant explanatory variables ($P < 0.05$) remained (McCullagh & Nelder 1989). We added each non-significant variable back into the model one-by-one to ensure that we did not overlook any significant effects. For all models, we used the 'glmmADMB' package (Fournier et al. 2012) within the R statistical software v 2.15.2 (R Development Core Team 2011), which permits simultaneous modeling of random effects, offsets, and an overabundance of zeroes in frequency data (i.e., zero inflation). Lastly, we used a linear regression (JMP 10.0) to test if males and females within a pair coordinated their rates (frequency/hour) of mark investigation and urogenital marking within an observational period.

Results

Sex Differences in the Chemistry of Genital Signals

We detected a rich array of 252 unique components in the genital secretions of Coquerel's sifakas that included a mixture of hydrocarbons, alcohols, acids, cholesterol derivatives, squalene, and fatty-acid esters. Of these compounds, 239 (94.8%) were expressed by both sexes (albeit by different individuals and in different relative abundances), whereas the remaining 13 (5.2%) were specific to one sex (as defined by presence in minimally two samples). Not surprisingly, therefore, the chromatograms of the secretions from males and females differed visually, even within the same season (Fig. 1a-b). We captured one aspect of this sex difference by PCA/LDA: Retaining only the 57 compounds that all individuals expressed and using the relative abundances of these shared compounds, we reduced the dimensionality of the dataset to 10 PCs that cumulatively explained 96.2% of the variation; LDA

of these PCs correctly classified the sex of 100% of the subjects. As predicted, despite a highly conservative approach based on less than 23% of the compounds detected, this analysis revealed significant sex differences in the abundance of shared chemicals in sifaka genital secretions (Wilks' $\lambda = 0.05$, $P < 0.05$: Fig. 1c).

Seasonal Differences in the Chemistry of Genital Signals

The chromatograms derived from female genital odorants were visually distinctive across the different seasons (Fig. 1b, 2a-b). For labial secretions, we extracted 5 PCs from the 27 compounds that were present across all female samples and all seasons and that cumulatively explained 90.1% of the variation. LDA analysis of these PCs correctly classified 100% of the female samples to the correct season and revealed a significant difference between the seasons (Wilks' $\lambda = 0.03$, $P < 0.0001$, Fig. 2c).

By contrast, the chemical analyses of male genital odorants revealed no clear seasonal pattern. Although male scrotal secretions shared 44 compounds across all samples and all seasons, LDA analysis of the 5 PCs that explained 89.4% of the variation misclassified the seasonal category of 21.1% of the male samples and revealed no significant seasonal difference in chemical composition (Wilks' $\lambda = 0.49$, $P = 0.46$).

Chemical Profiles and Social Bondedness

Although the chemical distance between dyad members did not correlate to the number of years the animals had cohabitated ($F_{1,6} = 2.01$, $R^2 = 25\%$, $P = 0.21$), there was a nonsignificant trend, in the predicted direction, for sifakas to be less chemically distant (i.e., smell more similar) if they had previously reproduced with one another than if they had never

reproduced with one another (Mann-Whitney $U = 13$, $N_1 = 5$, $N_2 = 3$, $P = 0.10$, one-tailed, Fig. 3).

Housing Effects on Olfactory Behaviour

Sifakas investigated marks and engaged in urogenital marking more frequently when they were housed in pens than when they were semi-free ranging in the forest; however, there was no effect of housing on the proportion of scent marks that were overmarks (see electronic Supplementary Material, Fig. S4). Because confinement increases certain olfactory behaviour, we considered concurrent housing condition in all subsequent behavioural analyses.

Sex Differences in Olfactory Behaviour

In sifakas, the sexes invested differently in olfactory behaviour: males investigated marks (GLMM: Z = 13.40, $P < 0.0001$) and scent marked (GLMM: Z = 2.88, $P < 0.005$) significantly more than did females (Fig. 4a). Moreover, relative to females, males placed a greater proportion of their urogenital marks over previously established marks (GLMM: Z = 5.08, $P < 0.0001$; Fig. 4b).

Seasonal Differences in Olfactory Behaviour

We also found significant seasonal variation in sifaka olfactory behaviour with the greatest activity generally occurring during the pre-breeding and breeding seasons. Specifically, the frequency of both mark investigation (GLMM: Z = -5.42, $P < 0.0001$) and urogenital marking (GLMM: Z = -2.31, $P < 0.05$) decreased in the post-breeding season relative to the pre-breeding and breeding seasons (Fig. 5a,b). Similarly, there was a seasonal decrease in the

proportion of urogenital overmarks, with overmarking decreasing in the breeding season relative to the pre-breeding season (GLMM: Z = -2.11, $P < 0.05$) and again in the post-breeding season relative to the breeding season (GLMM: Z = -2.88, $P < 0.005$).

Olfactory Coordination and Social Bondedness

Despite individual sex differences, male and female sifakas within breeding pairs coordinated their olfactory behaviour with one another, such that linear regressions on pairwise mark investigation ($F_{1,597} = 7.81$, $R^2 = 1.3\%$, $P < 0.01$) and urogenital marking ($F_{1,597} = 201.08$, $R^2 = 25\%$, $P < 0.0001$; Fig. 6a) revealed significant, positive correlations. For example, within an observational period, when one member of the pair increased its rate of urogenital marking, so too did its potential mate (Fig. 6b).

When we defined social bondedness by the number of years that the pair had been cohabitating, we found no effect of bond strength on mark investigation (GLMM: Z = 0.35, $P = 0.73$), urogenital marking (GLMM: Z = 1.26, $P = 0.21$) or overmarking (GLMM: Z = 1.04, $P = 0.30$). When we defined social bondedness by the pair's prior reproductive success, however, we found a significant effect of bond strength on sifaka olfactory behaviour. Specifically, as predicted, pairs that had been reproductively successful in the past, engaged less frequently in urogenital marking than did pairs that had never reproduced together (GLMM: Z = 4.72, $P < 0.0001$; Fig. 7a). Reproductively successful pairs also appeared to investigate marks less frequently than did reproductively unsuccessful pairs; however, this trend was not statistically reliable (GLMM: Z = 1.84, $P = 0.066$; Fig. 7a). Past reproductive history had no effect, however, on the proportion of urogenital overmarks (GLMM: Z = 0.19, $P = 0.85$; Fig. 7b).

Discussion

By integrating chemical analyses of glandular secretions produced in different phases of the reproductive season with observation of concurrent marking behaviour, we revealed a complex repertoire of olfactory communication in the sifaka. Consistent with the social complexity hypothesis for communication and our argument for convergent evolution, sifakas, like other diurnal and socially integrated strepsirrhines, rely heavily on glandular scent signalling. We found that their genital secretions alone contain a rich array of volatile chemicals, comparable in number and type to those expressed by members of Lemuridae (ring-tailed lemurs: Scordato et al. 2007; various *Eulemur* species: delBarco-Trillo et al. 2012). As many of these compounds are costly to synthesize, they are likely to be functionally significant (Krebs & Dawkins, 1984). Minimally, these compounds encode the sex and, in females, also the fertility of the signaller -- information crucial to coordinating reproduction (see also Morelli et al. 2013).

The patterns in the chemical matrix of sifaka genital-scent signals were further punctuated by observable patterns in their scent-marking behaviour. As in other mammalian taxa (Ralls 1971; Eisenberg & Kleiman 1972; Johnson 1973), the frequencies of scent investigation and scent deposition by sifakas were differentiated between the sexes and across reproductive seasons. Consistent with our social cohesion hypothesis, reproductive coordination between the sexes was reflected in olfactory behaviour that was both positively correlated between mates and influenced by the pair's reproductive history. Moreover, scent chemistry may have converged in established breeders, given their more similar scent signatures relative to pairs that had never reproduced. As in other social species (Rothman & Mech 1979; Porton 1983; Savage et al. 1988; Kranz 1991), olfactory communication in sifakas could allow potential mates to establish and maintain social bonds. Based on these findings, we suggest that chemical signalling represents a

potent communicatory modality that is influenced by a species' social complexity, with 'complexity' being defined, in part, by the quality of the social or reproductive bond between individuals.

Several methodological factors likely contributed to revealing these patterns. First, availability of six stable groups of comparable size and social structure throughout a two-year period provided the necessary comparative study population. Second, fairly consistent access to our subjects allowed the occasional animal handling necessary to coordinate chemical and behavioural sampling, and the routine visual proximity necessary to witness fine patterns in olfactory behaviour. Third, we profited from the detailed and long-term, life-history records available on our subjects that allowed assigning a narrower window of female fertility than previously feasible (e.g. a 6-week period vs. a 3-month 'breeding season': Brockman 1999; Lewis 2005; Pochron et al. 2005). Lastly, our use of the breeding pair as the focal unit was critical for detecting olfactory coordination between individuals.

Within breeding pairs, sifakas coordinated their olfactory behaviour by concurrently adjusting their frequency of scent investigation and scent deposition, and by overmarking their mate's marks. Like the synchronized vocal duets or choirs of many social species (Harrington 1989; Brumm & Slater 2007; Hall 2009; Zaccaroni et al. 2012), coordinated scent marking between sifaka mates could function to jointly defend territories or to advertise the pair's association. Scent overmarking also can be a competitive behaviour between males, used to defend resources (Ferkin 1999; Rich & Hurst 1999). In sifakas, scent overmarking is expressed primarily by males, who can cover up to 95% of female marks (Lewis 2005). The chemical contribution from the male's overmark could mix the pair's secretions and/or scramble the fertility information encoded in female signals. Although we have little understanding of the

chemical consequences of overmarking in any species, we found that sifaka overmarking progressively decreased in both sexes across the reproductive season.

Olfactory signalling in sifakas also varied by the quality of the social bond; however, bondedness in sifakas may reflect the importance of shared experiences more so than of shared time together. Notably, although we found no evidence that the tenure of a pair's social relationship predicted patterns in scent chemistry or olfactory behaviour, we did find evidence that a pair's reproductive history modestly predicted patterns in scent chemistry and significantly influenced olfactory behaviour. Relative to pairs that had produced offspring in the past, pairs that had not yet reproduced scent marked more frequently, potentially as an expensive 'getting-to-know-you' mechanism during the period of bond formation. Once this bond has solidified, as evidenced by successful reproduction, pair members may reduce their costs incurred via scent deposition.

Although limited by small sample sizes, sifakas in strongly bonded pairs tended to express more similar chemical profiles than individuals in weakly bonded pairs. We suggest that breeding pairs may converge their chemical profiles as their social bond strengthens. Given that kinship predicts scent similarity in lemurs (Charpentier et al. 2008, Boulet et al. 2009; Morelli et al. 2013) and is detectable by receivers (Charpentier et al. 2010), inbreeding avoidance would make it unlikely for scent similarity to predict reproductive success. Instead, as in social species that express group signatures in their odour cues (Bloss et al. 2002; Theis et al. 2012), signatures can be adopted by immigrating members (Safi & Kerth 2003). The function of signal convergence in supporting group or pair cohesion previously has been shown for vocal communication. In some primates (Geissmann & Orgeldinger 2000) and many avian taxa (Brumm & Slater 2007; Tyack 2008; Sewall 2009), vocal signals become more alike or more

complementary across the period of pair association or bond formation. During development, bat vocalizations become increasingly similar to colony vocal signatures (Knörnschild et al. 2012). Likewise, a primate's proximity to group members has been shown to influence similarities in vocal signals (Candiotti et al. 2012). Although the mechanism of vocal convergence implicates learning, the mechanism of olfactory convergence is likely to involve similar volatile production through shared microbial communities (Archie & Theis 2011; Sin et al. 2012; Theis et al. 2012). Accordingly, physical contact, including via allo-marking, overmarking, or sexual reproduction, likely contributes to the exchange of odorant-producing microbes. To the extent that scent signatures might converge in bonded pairs to signal similar messages, mated sifakas could further economize on their expenditure in advertisement.

The premise that the evolution of complex communicatory signals is linked to the evolution of sociality is heavily reliant on studies of vocal signals and on the use of group size as a proxy of social complexity. We further expand this evolutionary framework to include studies of olfactory signals and emphasize the usefulness of stable relationships or social bondedness as a metric of social complexity. Notably, we provide the first evidence that the strength of a social bond can be reflected in the signallers' olfactory behaviour and potentially also in the chemistry of its scent signals. This broader, yet unifying framework could profitably be used to examine semiochemistry and patterns of scent marking in other key species characterized by bonded relationships; however, future tests of the social complexity and social cohesion hypotheses would also benefit from a species-comparative approach.

Acknowledgments

This work was supported by Molly H. Glander Memorial Undergraduate Research Grants, Duke University Undergraduate Research Support grants, and a National Science Foundation REU (to L.K.G.), and by a National Science Foundation research grant IOS-0719003 (to C.M.D.). At the time of writing, both authors were supported by National Science Foundation research grant IOS-1021633 (to C.M.D.)

We thank the Duke Lemur Center staff, particularly D. Brewer, B. Schopler, J. Taylor, C. Williams, and S. Zehr, for facilitating sample collection. G. Dubay kindly provided access to the instrumentation for GC/MS and, with J.C. Crawford, provided training on its use, and help with interpreting the chemical data. We are grateful to the Statistical Consulting Center of Duke University and J. delBarco-Trillo for advice in data analysis. This is DLC publication # 1254.

560 **References**

561

562 **Alberts, A.C.** 1992. Constraints on the design of chemical communication systems in terrestrial
563 vertebrates. *The American Naturalist*, **139** (Supplement), S62-S69.

564 **Albone, E.S.** 1984. Microorganisms in mammalian semiochemistry. In: *Mammalian*
565 *semiochemistry* (Ed. by E.S. Albone), pp. 135-164. New York: John Wiley & Sons.

566 **Andrainarivo, C., Andriaholinirina, V.N., Feistner, A., Felix, T., Ganzhorn, J., Garbutt, N.,**
567 **Golden, C., Konstant, B., Louis Jr., E., Meyers, D., Mittermeier, R.A., Perieras, A.,**
568 **Princee, F., Rabarivola, J.C., Rakotosamimanana, B., Rasamimanana, H.,**
569 **Ratsimbazafy, J., Raveloarinoro, G., Razafimanantsoa, A., Rumpler, Y., Schwitzer,**
570 **C., Thalmann, U., Wilmé, L. & Wright, P**. 2008. *Propithecus coquereli*. In: IUCN
571 2012. IUCN Red List of Threatened Species. Version 2012.2. <www.iucnredlist.org>.
572 Downloaded on **12 June 2013**.

573 **Archie, E.A. & Theis, K.R.** 2011. Animal behaviour meets microbial ecology. *Animal*
574 *Behaviour*, **82**, 425-436.

575 **Barrett, L. & Henzi, P.** 2005. The social nature of primate cognition. *Proceedings of the Royal*
576 *Society B*, **272**, 1865-1875.

577 **Becker, E.A., Petruno, S. & Marler, C.A.** 2012. A comparison of scent marking between a
578 monogamous and promiscuous species of *Peromyscus*: pair bonded males do not
579 advertise to novel females. *PLoS ONE*, **7,** e32002.

580 **Bekoff, M. 1979.** Scent marking by free ranging domestic dogs: olfactory and visual
581 components. *Biology of Behaviour*, **4**, 123-129

582 **Bloss, J., Arcee, T.E., Bloss, J.M., Hood, W.R. & Kunz, T.H.** 2002. Potential use of chemical
583 cues for colony-mate recognition in the brown bat, *Eptesicus fuscus*. *Journal of Chemical*
584 *Ecology*, **28**, 819-834.
585 **Boulet, M., Charpentier, M.J.E. & Drea, C.M.** 2009. Decoding an olfactory mechanism of kin
586 recognition and inbreeding avoidance in a primate. *BMC Evolutionary Biology*, **9**, 281.
587 **Boulet, M., Crawford, J.C., Charpentier, M.J.E. & Drea, C.M.** 2010. Honest olfactory
588 ornamentation in a female-dominant primate. *Journal of Evolutionary Biology*, **23**, 1558-
589 1563.
590 **Brockman, D.K.** 1999. Reproductive behavior of female *Propithecus verreauxi* at Beza
591 Mahafaly, Madagascar. *International Journal of Primatology*, **20**, 375-398.
592 **Brumm, H. & Slater, P. 2007.** Animal communication: timing counts. *Current Biology*, **17**,
593 R521-R523.
594 **Buesching, C.D., Stopka, P. & Macdonald, D.W.** 2003. The social function of allo-marking in
595 the European badger (*Meles meles*). *Behaviour*, **140**, 965-980.
596 **Butler, R.G. & Butler, L.A.** 1979. Toward a functional interpretation of scent marking in the
597 beaver (*Castor canadensis*). *Behavior and Neural Biology*, **26**, 442-454.
598 **Byrne, R.W. & Whiten, A.** 1988. *Machiavellian Intelligence: Social Expertise and the*
599 *Evolution of Intellect in Monkeys, Apes and Humans*. Oxford: Clarendon Press.
600 **Candiotti, A., Zuberbühler, K. & Lemasson, A.** 2012. Convergence and divergence in Diana
601 monkey vocalizations. *Biology Letters*, **8**, 382-385.
602 **Charpentier, M.J.E., Boulet, M. & Drea, C.M.** 2008. Smelling right: the scent of male lemurs
603 advertises genetic quality and relatedness. *Molecular Ecology*, **17**, 3225-3233.
604 **Charpentier, M.J.E., Crawford, J.C., Boulet, M. & Drea, C.M.** 2010**.** Message 'scent':

lemurs detect the genetic relatedness and quality of conspecifics via olfactory cues. *Animal Behaviour*, **80**, 101-108.

Cheney, D., Seyfarth, R. & Smuts, B. 1986. Social relationships and social cognition in nonhuman primates. *Science*, **234**, 1361-1366.

Clarke, P.M.R, Barrett, L. & Henzi, S.P. 2009. What role do olfactory cues play in Chacma baboon mating? *American Journal of Primatology*, **71**, 493-502.

delBarco-Trillo, J., Burket, B.A., Goodwin, T.E. & Drea, C.M. 2011. Night and day: the comparative study of strepsirrhine primates reveals socioecological and phylogenetic patterns in olfactory signals. *Journal of Evolutionary Biology*, **24**, 82-98.

delBarco-Trillo, J., Sacha, C.R., Dubay, G.R. & Drea, C.M. 2012. *Eulemur*, me lemur: the evolution of scent-signal complexity in a primate clade. *Philosophical Transactions of the Royal Society B*, **367**, 1909-1922.

De Waal, F. B. M. & Tyack, P. L. 2003. *Animal Social Complexity*. Harvard, MA: Harvard University Press.

Drea, C.M. & Scordato, E.S. 2008. Olfactory communication in the ringtailed lemur (*Lemur catta*): form and function of multimodal signals. In: *Chemical Signals in Vertebrates 11* (Ed. By J.L. Hurst, R.J. Beynon, S.C. Roberts & T.D. Wyatt), pp. 921-102. New York: Springer.

Drea, C.M., Boulet, M., delBarco-Trillo, J., Greene, L.K., Sacha, C.R., Goodwin, T.E. & Dubay, G.R. 2013. The "secret" in secretions: methodological considerations in deciphering primate olfactory communication. *American Journal of Primatology*, **75**, 621-642.

627 **Dunbar, R.I.M.** 1993. Coevolution of neocortical size, group size and language in humans.
628 *Behavioral and Brain Sciences*, **16**, 681-735.
629 **Dunbar, R.I.M** 1998. The social brain hypothesis. *Evolutionary Anthropology*, **6**, 178-190.
630 **Dunbar, R.I.M & Schultz, S.** 2007. Evolution in the social brain. *Science*, **317**, 1344-1347.
631 **Eisenberg, J.F. & Kleiman, D.G.** 1972. Olfactory communication in mammals. *Annual Review*
632 *of Ecology and Systematics*, **3**, 1-32.
633 **Erkert, H.G. & Kappeler, P.M.** 2004. Arrived in the light: diel and seasonal activity patterns in
634 wild Verreaux's sifakas (*Propithecus v. verreauxi*; Primates: Indriidae). *Behavioral*
635 *Ecology and Sociobiology*, **57**, 174-186.
636 **Estes, R.D. & Goddard, J.** 1967. Prey selection and hunting behavior of the African wild dog.
637 *The Journal of Wildlife Management*, **31**, 52-70.
638 **Ferkin, M.H.** 1999. Meadow voles (*Microtus pennsylvanicus*, Arvicolidae) over-mark and
639 adjacent-mark the scent marks of same-sex conspecifics. *Ethology*, **105**, 825-837.
640 **Fournier, D.A., Skaug, H.J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M.N.,**
641 **Nielsen, A. & Sibert, J.** AD Model Builder: using automatic differentiation for statistical
642 inference of highly parameterized complex nonlinear models. *Optimization Methods and*
643 *Software*, **27**, 233-249.
644 **Freeberg, T.M.** 2006. Social complexity can drive vocal complexity: group size influences
645 vocal information in Carolina chickadees. *Physiological Science*, **17**, 557-561.
646 **Freeberg, T.M. & Lucas, J.R.** 2012. Information theoretical approaches to chick-a-dee calls of
647 Carolina chikadees (*Poecile carolinensis*). *Journal of Comparative Psychology*, **126**, 68-
648 81.

649 **Freeberg. T.M., Dunbar, R.I.M. & Ord, T.J.** 2012. Social complexity as a proximate and
650 ultimate factor in communicative complexity. *Philosophical Transactions of the Royal*
651 *Society B*, **367**, 1785-1801.
652 **Geissmann, T. & Orgeldinger, M.** 2000. The relationship between duet songs and pair bonds in
653 siamangs, *Hylobates syndactylus*. *Animal Behaviour*, **60**, 805-809.
654 **Glickman, S.E., Zabel, C.J, Yoerg, S.I., Weldele, M.L., Drea, C.M. & Frank, L.G.** 1997.
655 Social facilitation, affiliation, and dominance in the social life of spotted hyenas. *Annals*
656 *of the New York Academy of Sciences*, **807**, 175-184.
657 **Gosling, L.M., Roberts, S.C., Thornton, E.A. & Andrew, M.J.** 2000. Life history costs of
658 olfactory status signalling in mice. *Behavioral Ecology and Sociobiology*, **48**, 328-332.
659 **Hall, M.L.** 2009. A review of vocal duetting in birds. *Advances in the Study of Behavior*, **40**, 67-
660 121.
661 **Harrington, F.H.** 1989. Chorus howling by wolves: acoustic structure, pack size and the Beau
662 Geste effect. *Bioacoustics*, **2**, 117-136.
663 **Harrington, J.E.** 1979. Responses of *Lemur fulvus* to scents of different subspecies of *L. fulvus*
664 and to the scents of different species of Lemuriformes. *Zeitschrift für Tierpsychologie*,
665 **49**, 1-9.
666 **Horvath, J.E., Weisrock, D.W., Embry, S.L., Fiorentino, I., Balhoff, J.P., Kappeler, P.,**
667 **Wray, G.A., Willard, H.F. & Yoder, A.D.** 2008. Development and application of a
668 phylogenomic toolkit: resolving the evolutionary history of Madagascar's lemurs.
669 *Genome Research*, **18**, 489-499.
670 **Johnson, R.P.** 1973. Scent marking in mammals. *Animal Behaviour*, **21**, 521-535.

671 **Jordan, N.R., Cherry, M.I. & Manser, M.B.** 2007. Latrine distribution and patterns of use by
672 wild meerkats: implications for territory and mate defense. *Animal Behaviour*, **73**, 613-
673 622.
674 **Kappeler, P.M. & Schäffler, L.** 2008. The lemur syndrome unresolved: extreme male
675 reproductive skew in sifakas (*Propithecus verreauxi*), a sexually monomorphic primate
676 with female dominance. *Behavioral Ecology and Sociobiology*, **62**, 1007-1015.
677 **Kleiman, D.G.** 1981. Correlations among life history characteristics of mammalian species
678 exhibiting two extreme forms of monogamy. In: *Natural Selection and Social Behavior*
679 (Ed. By R.D. Alexander & D.W. Tinkle), pp. 332-344, New York: Chiron Press.
680 **Knörnschild, M., Nagy, M., Metz, M., Mayer, F & von Helversen, O.** 2012. Learned vocal
681 group signatures in the polygynous bat *Saccopteryx bilineata*. *Animal Behaviour*, **84**,
682 761-769.
683 **Kranz, K.R.** 1991. Monogamy in the dik-dik. *Applied Animal Behaviour Science*, **29**, 87-105.
684 **Kraus, C., Heistermann, M. & Kappeler, P.M.** 1999. Physiological suppression of sexual
685 function in subordinate males: a subtle form of intrasexual competition among male
686 sifakas (*Propithecus verreauxi*)? *Physiology & Behavior*, **66**, 855-861.
687 **Krebs, J.R. & Dawkins, R.** 1984. Animal signals: mind-reading and manipulation. *Behavioural*
688 *Ecology: an evolutionary approach*, ***2***, 380-402.
689 **Kruuk, H.** 1972. *The Spotted Hyena: a Study of Predation and Social Behavior.* Chicago:
690 University of Chicago Press.
691 **Kubzdela, K.S., Richard, A.F. & Pereira, M.E.** 1992. Social relations in semi-free-ranging
692 sifakas (*Propithecus verreauxi coquereli*) and the question of female dominance.
693 *American Journal of Primatology*, **28**, 139-145.

694 **Lenochova, P., Roberts, S.C. & Havlicek, J.** 2008. Methods of human odor sampling: the
695 effects of freezing. *Chemical Senses*, **34**, 127-138.
696 **Lewis, R.J.** 2005. Sex differences in scent-marking in sifaka: mating conflict or male services?
697 *American Journal of Physical Anthropology*, **128**, 389-398.
698 **Lewis, R.J.** 2008. Social influences on group membership in *Propithecus verreauxi verreauxi*.
699 *International Journal of Primatology*, **29**, 1249-1270.
700 **Maestripieri, D.** 2005. Gestural communication in three species of macaques (*Macaca mulatta,*
701 *M. nemestrina, M. arctoides). Gesture*, **5**, 57-73.
702 **Martin, P. & Bateson, P.** 1993. *Measuring Behaviour*. Cambridge: Cambridge University Press.
703 **McCullagh, P. & Nelder, J.A.** 1989. *Generalized Linear Models*. 2nd edn. London: Chapman
704 and Hall.
705 **McComb, K. & Semple, S.** 2005. Coevolution of vocal communication and sociality in
706 primates. *Biology Letters*, **1**, 281-385.
707 **McCune, B. & Grace, J.B.** 2002. *Analysis of Ecological Communities.* Gleneden Beach,
708 Oregon: MjM Software.
709 **Mertl-Millhollen, A.S.** 1979. Olfactory demarcation of territorial boundaries by a primate-
710 *Propithecus verreauxi*. *Folia Primatologica*, **32**, 35-42.
711 **Mertl-Millhollen, A.S., Goodmann, P.A. & Klinghammer, E.** 1986. Wolf scent marking with
712 raised-leg urination. *Zoo Biology*, **5**, 7-20.
713 **Morelli, T.L., Hayes, A.R., Nahrung, H.F., Goodwin, T.E., Harelimana, I.H., Macdonald,**
714 **L.J. & Wright, P.C.** 2013. Relatedness communicated in lemur scent.
715 *Naturwissenchaften*, **100**, 769-777.

716 **Overdorff, D.J. & Tecot, S.R.** 2007. Social pair-bonding and resource defense in wild red-
717 bellied lemurs (*Eulemur rubriventer*). In: *Lemurs: Ecology and adaptation* (Ed. by L.
718 Gould & M.L. Sauther), pp. 235-245. New York: Springer.
719 **Palagi, E., Telara, S. & Borgognini Tarli, S.M.** 2004. Reproductive strategies in *Lemur catta*:
720 balance among sending, receiving, and countermarking scent signals. *International*
721 *Journal of Primatology*, **25**, 1019-1031.
722 **Partan, S.R. & Marler, P.** 2005. Issues in the classification of multimodal communication
723 signals. *The American Naturalist*, **166**, 231-245.
724 **Pochron, S.T., Morelli, T.L., Scirbona, J. & Wright, P.C.** 2005. Sex differences in scent
725 marking in *Propithecus edwardsi* of Ranomafana National Park, Madagascar. *American*
726 *Journal of Primatology*, **66**, 97-110.
727 **Pollard, K.A. & Blumstein, D.T.** 2011. Social group size predicts the evolution of individuality.
728 *Current Biology*, **21**, 413-417.
729 **Porton, I.** 1983. Bush dog urine-marking: its role in pair formation and maintenance. *Animal*
730 *Behaviour*, **31**, 1061-1069.
731 **R Development Core Team**. 2011. R: *A Language and Environment for Statistical Computing.*
732 Vienna: R Foundation for Statistical Computing**.**
733 **Ralls, K.** 1971. Mammalian scent marking. *Science*, **171**, 443-449.
734 **Rich, T.J. & Hurst, J.L.** 1999. The competing countermarks hypothesis: reliable assessment of
735 competitive ability by potential mates. *Animal Behaviour*, **58**, 1027-1037.
736 **Richard, A.F., Rakotomanga, P. & Schwartz, M.** 1993. Dispersal by *Propithecus verreauxi* at
737 Beza Mahafaly, Madagascar: 1984-1991. *American Journal of Primatology*, **30**, 1-20.

738 **Richard, A.F., Dewar, R.E., Schwartz, M. & Ratsirarson, J.** 2002. Life in the slow lane?
739 Demography and life histories of male and female sifaka (*Propithecus verreauxi*
740 *verreauxi*). *Journal of Zoology*, **256**, 421-436.
741 **Roberts, S.C., Gosling, L.M., Thornton, E.A. & McClung, J.** 2001. Scent-marking by male
742 mice under risk of predation. *Behavioral Ecology*, **12**, 698-705.
743 **Rothman, R.J. & Mech, D.** 1979. Scent-marking in lone wolves and newly formed pairs.
744 *Animal Behaviour*, **27**, 750-760.
745 **Safi, K. & Kerth, G.** 2003. Secretions of the interaural gland contain information about
746 individuality and colony membership in the Bechstein's bat. *Animal Behaviour*, **65**, 363-
747 369.
748 **Savage, A., Ziegler, T.E. & Snowdon, C.T.** 1988. Sociosexual development, pair bond
749 formation, and mechanisms of fertility suppression in female cotton-top tamarins
750 (*Sanguinus oedipus oedipus*). *American Journal of Primatology*, **14**, 345-359.
751 **Schilling, A.** 1979. Olfactory communication in prosimians. In: *The Study of Prosimian*
752 *Behavior* (Ed. by R.D. Martin, G.A. Doyle & A.C. Walker), pp. 461-542. New York:
753 Academic Press.
754 **Schilling, A.** 1980. The possible role of urine in territoriality of some nocturnal prosimians.
755 *Symposia of the Zoological Society of London*, **45**, 165–193.
756 **Schultz, S., Opie, C. & Atkinson, Q.D.** 2011. Stepwise evolution of stable sociality in primates.
757 *Nature*, **479**, 219-224.
758 **Scordato, E.S. & Drea, C.M.** 2007. Scents and sensibility: information content of olfactory
759 signals in the ringtailed lemur, *Lemur catta*. *Animal Behaviour*, **73**, 301-314.

760 **Scordato, E.S., Dubay, G. & Drea, C.M.** 2007. Chemical composition of scent marks in the
761 ringtailed lemur (*Lemur catta*): glandular differences, seasonal variation, and individual
762 signatures. *Chemical Senses*, **32**, 493-504.
763 **Sewall, K.B.** 2009. Limited adult vocal learning maintains call dialects but permits pair-
764 distinctive calls in red crossbills. *Animal Behaviour*, **77**, 1303-1311.
765 **Silk, J.B.** 2007. The adaptive value of sociality in mammalian groups. *Philosophical*
766 *Transactions of the Royal Society B*, **362**, 539-559.
767 **Sin, Y.W., Buesching, C.D., Burke, T. & Macdonald, D.W.** 2012. Molecular characterization
768 of the microbial communities in the subcaudal gland secretion of the European badger
769 (*Meles meles*). *FEMS Microbiological Ecology*, **81**, 648-659.
770 **Sokal, R.R. & Rohlf, F.J.** 1995. *Biometry.* New York: W.H. Freeman.
771 **Theis, K.R., Schmidt, T.M. & Holekamp, K.E.** 2012. Evidence for a bacterial mechanism for
772 group-specific social odors among hyenas. *Scientific Reports*, **2**, 1-8.
773 **Tibbetts, E.A.** 2004. Complex social behaviour can select for variability in visual features: a
774 case study in *Polistes* wasps. *Proceedings of the Royal Society B*, **271**, 1955-1960.
775 **Tyack, P.L.** 2008. Convergence of calls as animals form social bonds, active compensation for
776 noisy communication channels, and the evolution of vocal learning in mammals. *Journal*
777 *of Comparative Psychology*, **122,** 319-331.
778 **Zaccaroni, M., Passilongo, D., Buccianti, A., Dessi-Fulgheri, F., Facchini, C., Gazzola, A.,**
779 **Maggini, I. & Apollonio, M.** 2012. Group specific vocal signature in free-ranging wolf
780 packs. *Ethology Ecology & Evolution***, 24**, 322-331.
781 **Zuur, A.F., Saveliev, A.A. & Leno, E.N.** 2012. *Zero Inflated Models and Generalized linear*
782 *Mixed Models with R.* Newburgh, United Kingdom: Highland Statistics Ltd.
783

784 **Figure legends**

785

786 **Figure 1.** Sex differences in sifaka genital secretions. Shown in (a, b) are representative
787 chromatograms of (a) male and (b) female secretions in the pre-breeding season. Dark arrows
788 point to the internal standard, hexachlorobenzene, added to the sample before the GC/MS run,
789 and light arrows point to the endogenously produced standard, squalene. Shown in (c) is a linear
790 discriminant (LD) plot of the principal components of the secretions for each sex across all
791 seasons. The LD analysis compares the relative abundances of shared compounds expressed by
792 males (black circles) and females (white circles; Wilks' λ, $P < 0.05$).

793

794 **Figure 2.** Seasonal differences in sifaka labial secretions. Shown in (a, b) are representative
795 chromatograms of the same female in the (a) breeding and (b) post-breeding seasons. Dark
796 arrows point to the internal standard, hexachlorobenzene, added to the sample before the GC/MS
797 run and light arrows point to the endogenously produced standard, squalene. Shown in (c) is a
798 linear discriminant (LD) plot of the principal components of the secretions for all females in each
799 season. The LD analysis compares the relative abundances of shared compounds expressed
800 during the pre-breeding (grey diamonds), breeding (black triangles), and post-breeding (white
801 squares) seasons (Wilks' λ, $P < 0.0001$).

802

803 **Figure 3.** Pairwise chemical distance between male-female dyads that either had no history of
804 past reproductive success ('RS,' black bar), or had previously reproduced together (white bar)
805 (Mann-Whitney *U*-test: § $P = 0.10$).

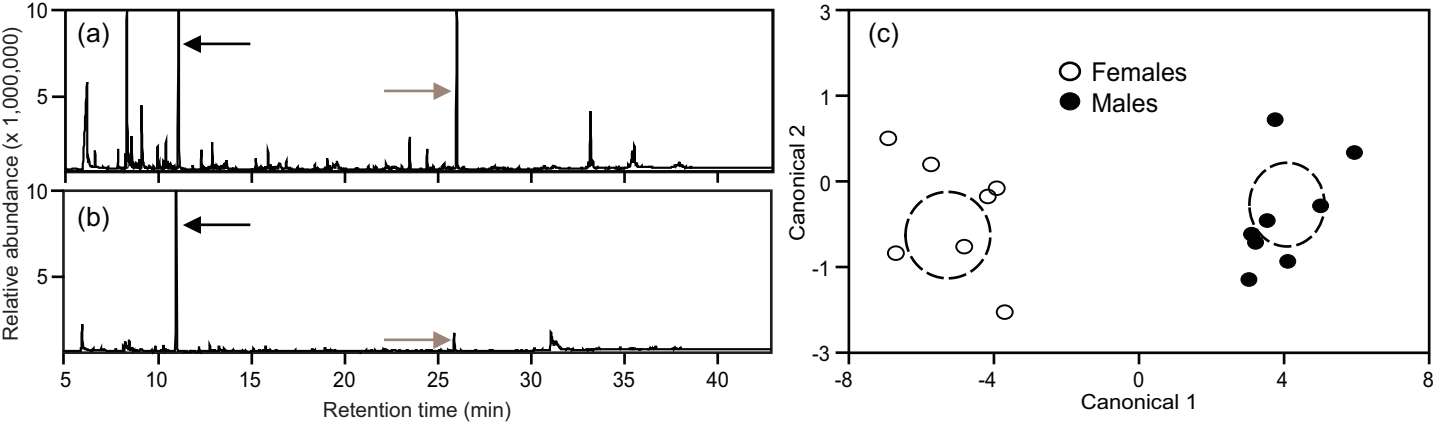
806

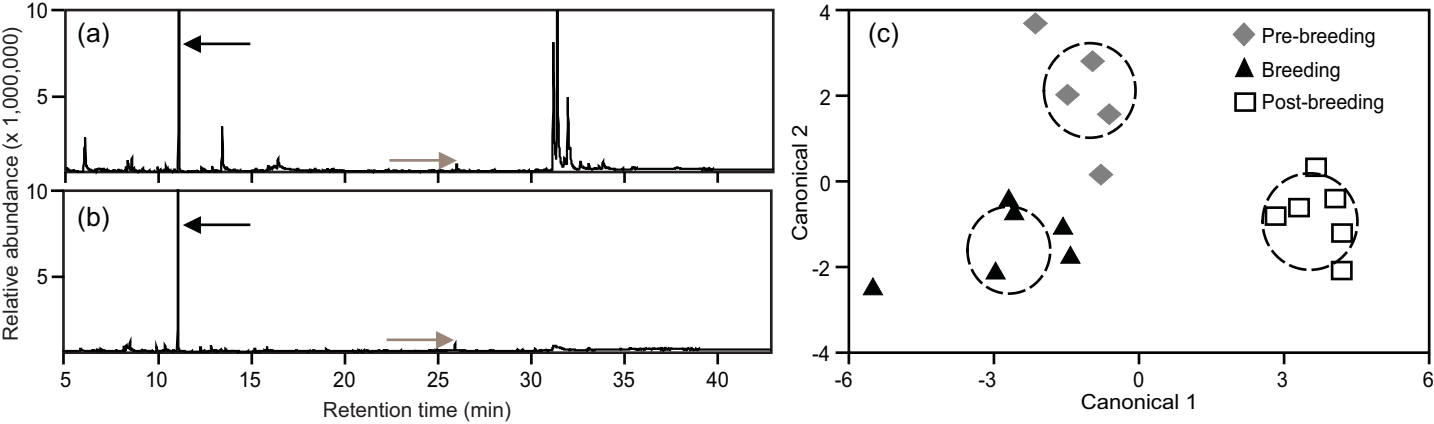
807 **Figure 4.** Sex differences in sifaka olfactory behaviour, including (a) the frequency of mark
808 investigation and urogenital marking, as well as (b) the proportion of overmarking in males
809 (black bars) and females (white bars) (GLMM: $^{**}P < 0.01$, $^{***}P < 0.001$).
810
811 **Figure 5.** Differences in sifaka olfactory behaviour in relation to female fertility (or reproductive
812 seasons). Shown is (a) the frequency of mark investigation by all animals, (b) the frequency of
813 urogenital marking by a representative female and male, and (c) the proportion of overmarking
814 by all animals in the pre-breeding (grey bars), breeding (black bars), and post-breeding (white
815 bars) seasons (GLMM: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).
816
817 **Figure 6**. Pairwise coordination in rates (frequency/hour) of urogenital marking depicted for all
818 observations of (a) each breeding pair (linear regression: $P < 0.0001$) across both study years and
819 (b) a representative breeding pair (male: black circles; female: white circles) shown sequentially
820 across the reproductive seasons of year 2. Each data point represents the rate of scent marking
821 during one observation period.
822
823 **Figure 7**. The olfactory behaviour of individual sifakas in relation to their dyad's reproductive
824 history. Shown are the frequencies of (a) mark investigation and (b) urogenital marking, as well
825 as the (c) proportion of overmarking. Black bars represent individuals living in pairs that had no
826 past reproductive success (RS); white bars represent individuals living in pairs that had past RS
827 (GLMM: § $P < 0.10$, $^{***}P < 0.001$).

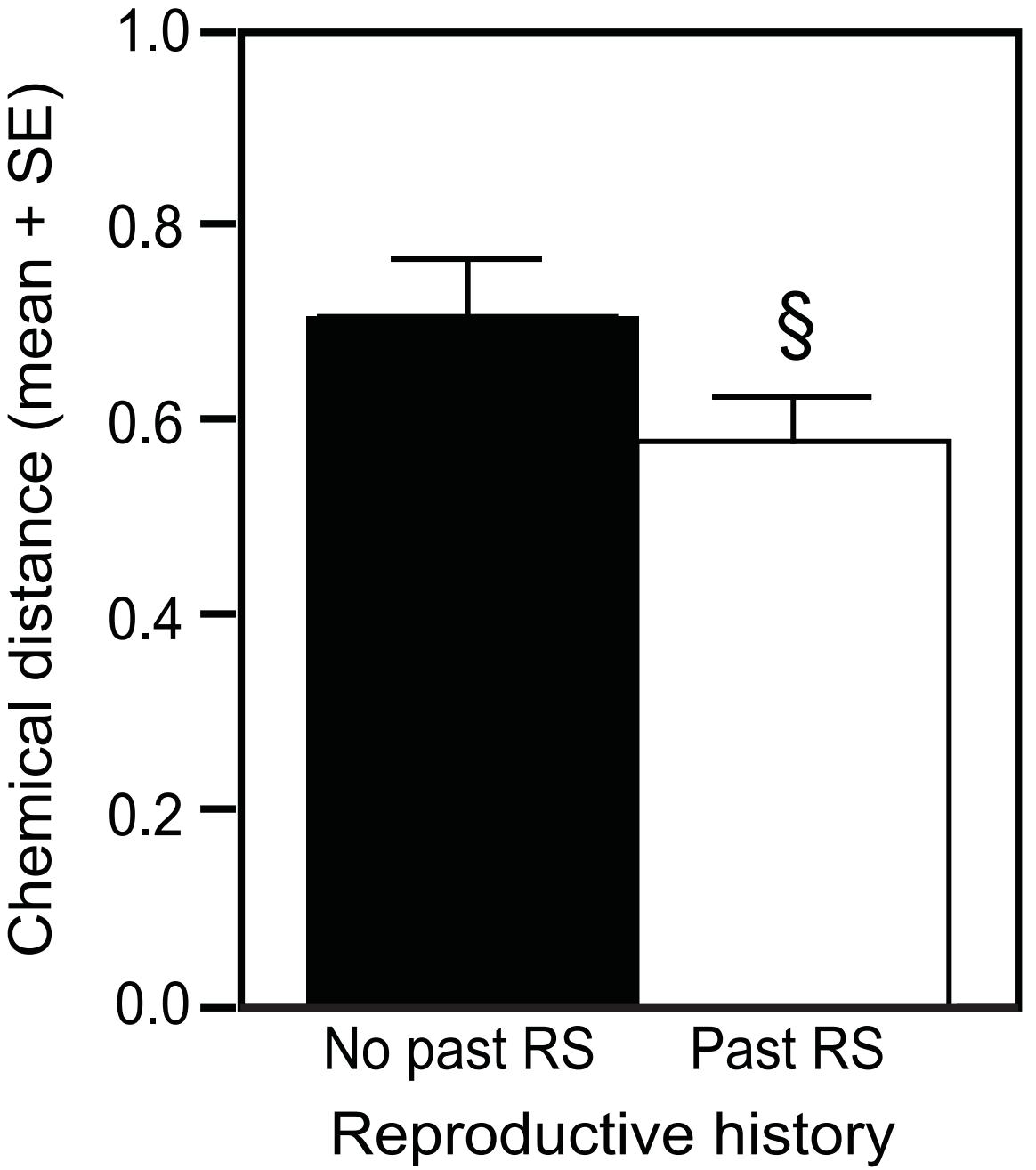
Table 1
Study subjects

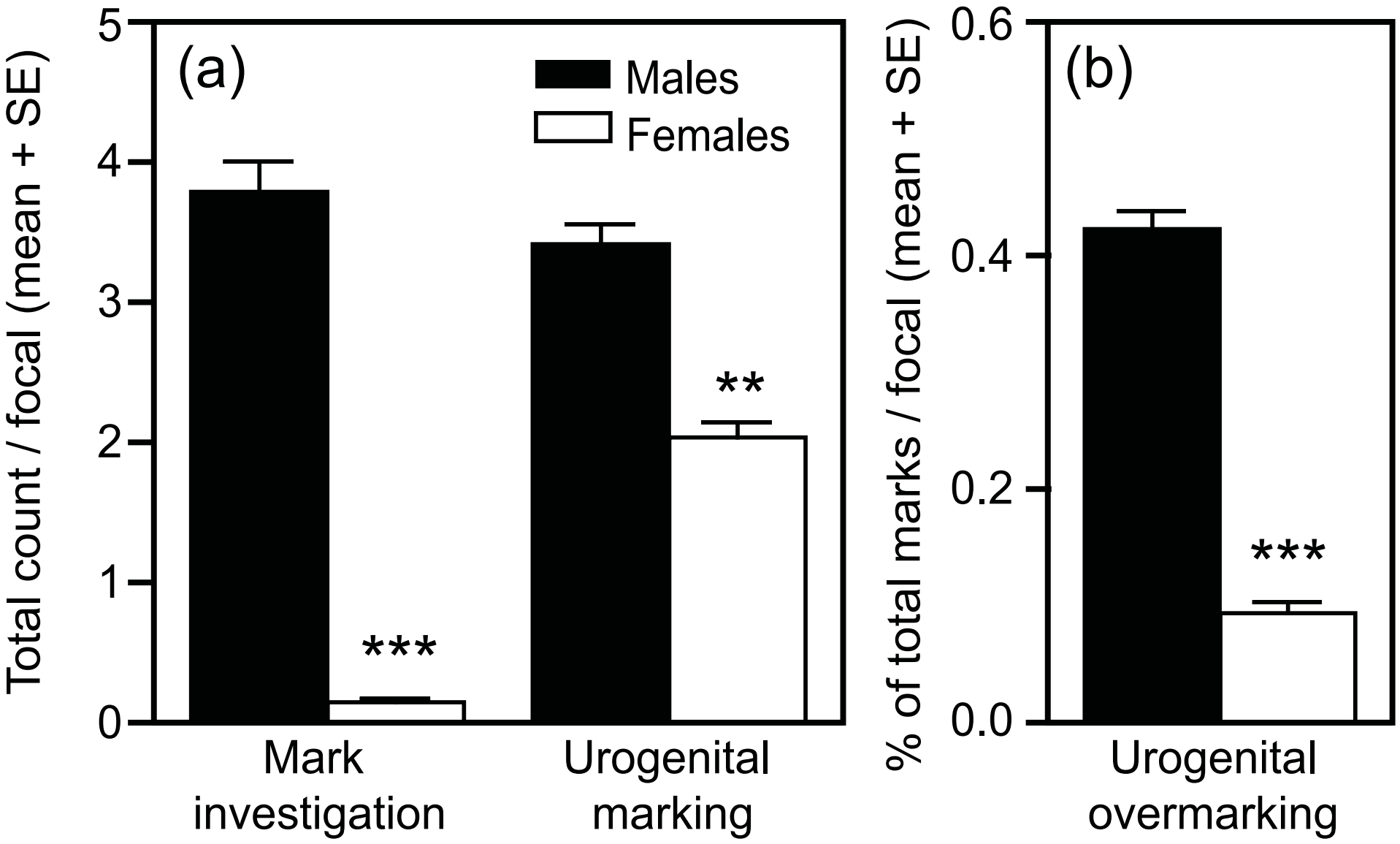
Group	Group size		Breeding pair					Subordinate adults	
			Age (yr)[a]		Duration of cohabitation by year 1 (yr)	Prior reproductive success[c]		Number & Sex	Age (yr)[a]
	Year 1	Year 2	Female[b]	Male[b]		Year 1	Year 2		
A	3	3	13	15	1	No	No	0	
D	4	5	17	17	9	Yes	Yes	0	
H	3	3	3	6	2	No	Yes	1 M	7
M	2	2	2	5	1	No	No	0	
P	5	6	11	16	10	Yes	Yes	1 M	3
R	5	5	11	22	2	Yes	Yes	1 F	4

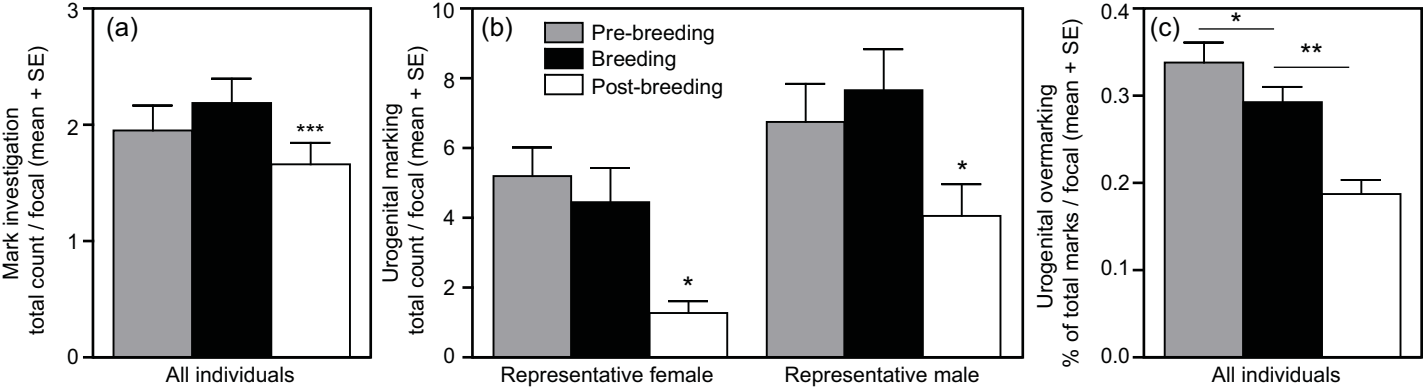
[a]Ages are provided in year 1 of the study.
[b]Female sifakas can cycle and successfully reproduce in their third year; males can successfully copulate by 3 years (Richard et al. 2002).
[c]Reproductive success is defined as having produced minimally one offspring together.

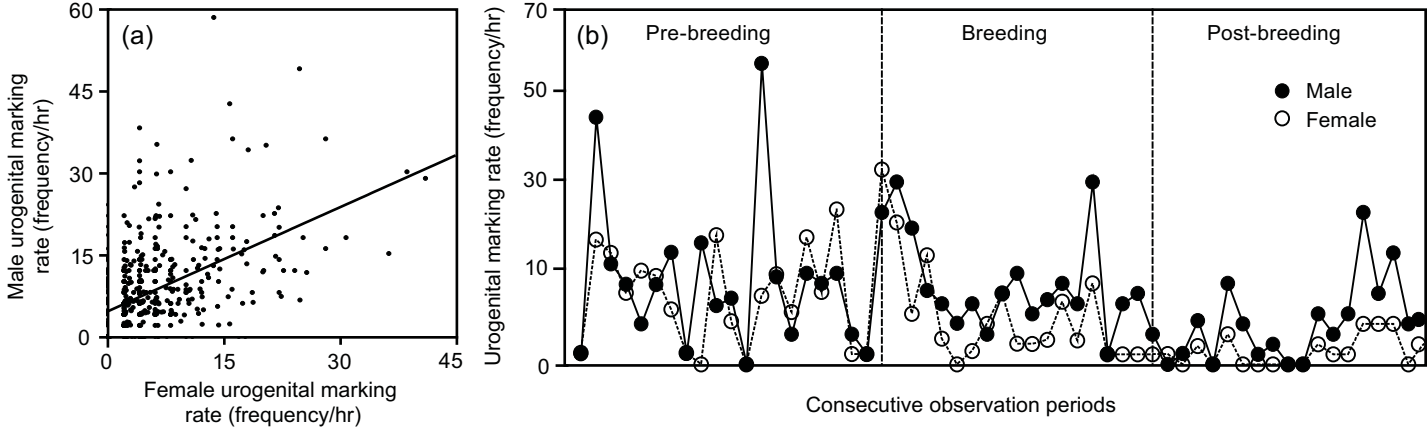

(a)
(b)
(c)
Relative abundance (x 1,000,000)
Retention time (min)
Canonical 1
Canonical 2
Females
Males

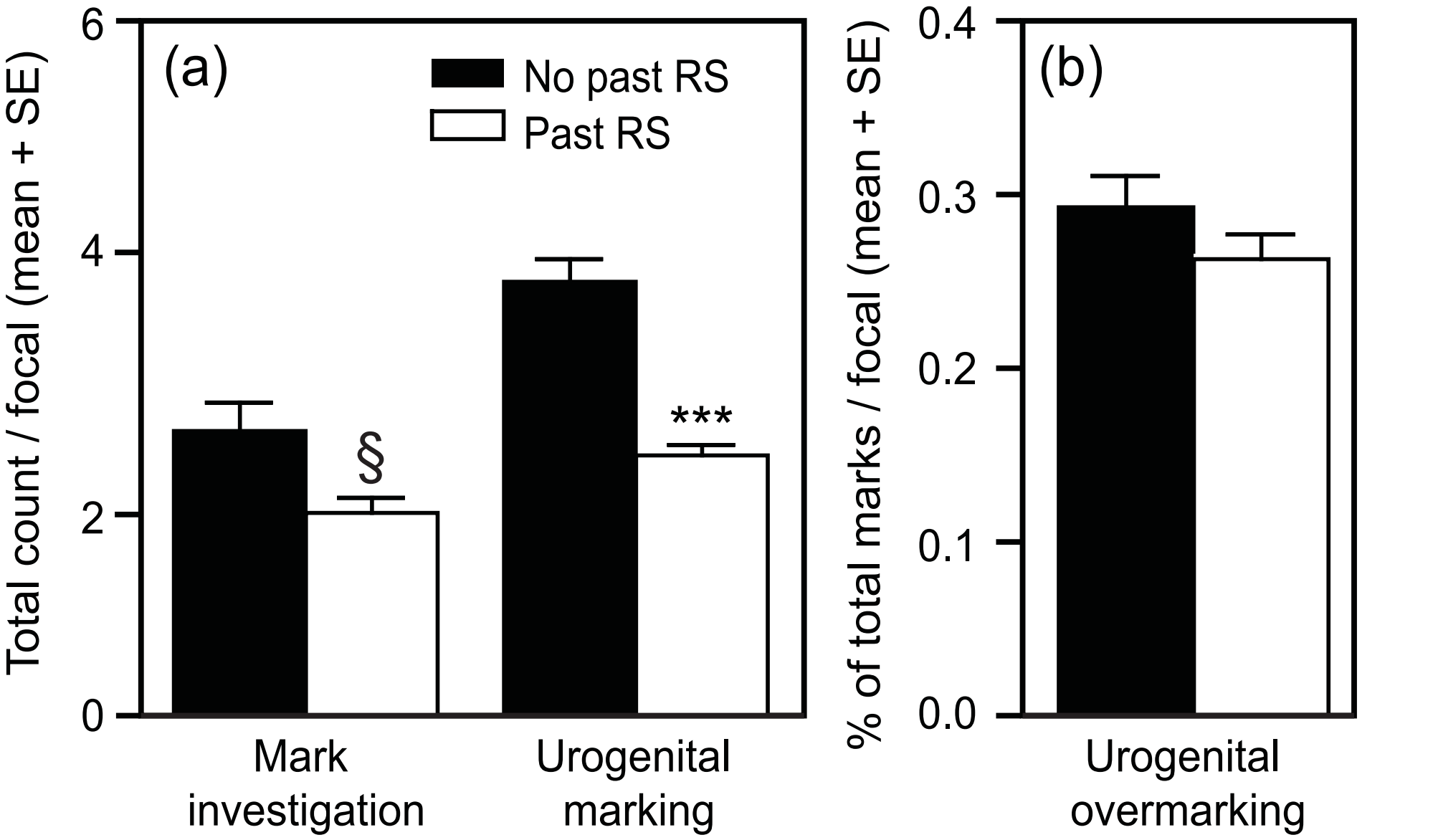

(a)
(b)
(c)
Relative abundance (x 1,000,000)
Retention time (min)
Canonical 1
Canonical 2
Pre-breeding
Breeding
Post-breeding


1.0
0.8
0.6
0.4
0.2
0.0
Chemical distance (mean + SE)
§
No past RS
Past RS
Reproductive history


(a)
Males
Females
Total count / focal (mean + SE)
5
4
3
2
1
0
***
**
Mark investigation
Urogenital marking
(b)
% of total marks / focal (mean + SE)
0.6
0.4
0.2
0.0
***
Urogenital overmarking


(a)
Mark investigation
total count / focal (mean + SE)
3
2
1
0
***
All individuals
(b)
Pre-breeding
Breeding
Post-breeding
Urogenital marking
total count / focal (mean + SE)
10
8
6
4
2
0
*
*
Representative female
Representative male
(c)
Urogenital overmarking
% of total marks / focal (mean + SE)
0.4
0.3
0.2
0.1
0.0
*
**
All individuals


(a)
Male urogenital marking rate (frequency/hr)
Female urogenital marking rate (frequency/hr)
0
15
30
45
60
(b)
Pre-breeding
Breeding
Post-breeding
Male
Female
Urogenital marking rate (frequency/hr)
Consecutive observation periods
0
10
30
50
70


(a)
No past RS
Past RS
Total count / focal (mean + SE)
6
4
2
0
§
***
Mark investigation
Urogenital marking
(b)
% of total marks / focal (mean + SE)
0.4
0.3
0.2
0.1
0.0
Urogenital overmarking

Supplementary Material

Figure S1. A male Coquerel's sifaka engaged in urogenital marking.

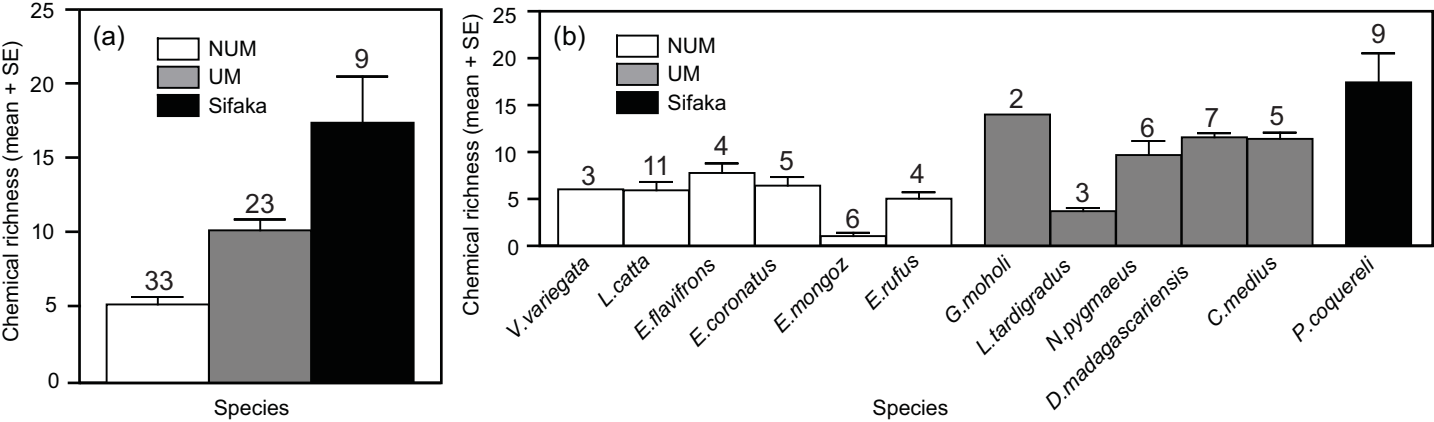
Figure S2. The number of compounds (i.e., chemical richness) expressed in the urinary signals of various strepsirrhine primates, including species that are categorized as primarily glandular markers or 'non-urine markers' (NUM: white bars), species that are categorized as primarily 'urine markers' (UM: grey bars), and sifakas that rely on both glandular and urinary signals (black bars). Shown here are the averages for (a) each category, plus sifakas, and (b) each species. In the original study by delBarco-Trillo and colleagues (2011), the number of compounds expressed by NUM was compared to that expressed by UM, with sifakas being subsumed into the latter UM category. Briefly, voided urine samples were collected from 65 adult, reproductively intact strepsirrhines, including 33 individuals from 6 NUM species, and 32 individuals from 6 UM species housed at the Duke Lemur Center, in Durham, NC. The headspace from each sample was analyzed for volatile compounds using solid-phase dynamic extraction (SPDE) coupled with GC/MS (see delBarco-Trillo et al. 2011 for full methods). For our present analysis, we retained the chemical richness (i.e., number of compounds) expressed in each sample, but divided the UM category into sifakas vs. all other UM individuals. The NUM category remained intact. We then calculated the mean chemical richness for each species and used those averages to calculate means and standard deviations (SD) for each marking type (i.e., UM, NUM, sifaka) using the JMP 10 statistical software package (SAS institute, 10.0.0).

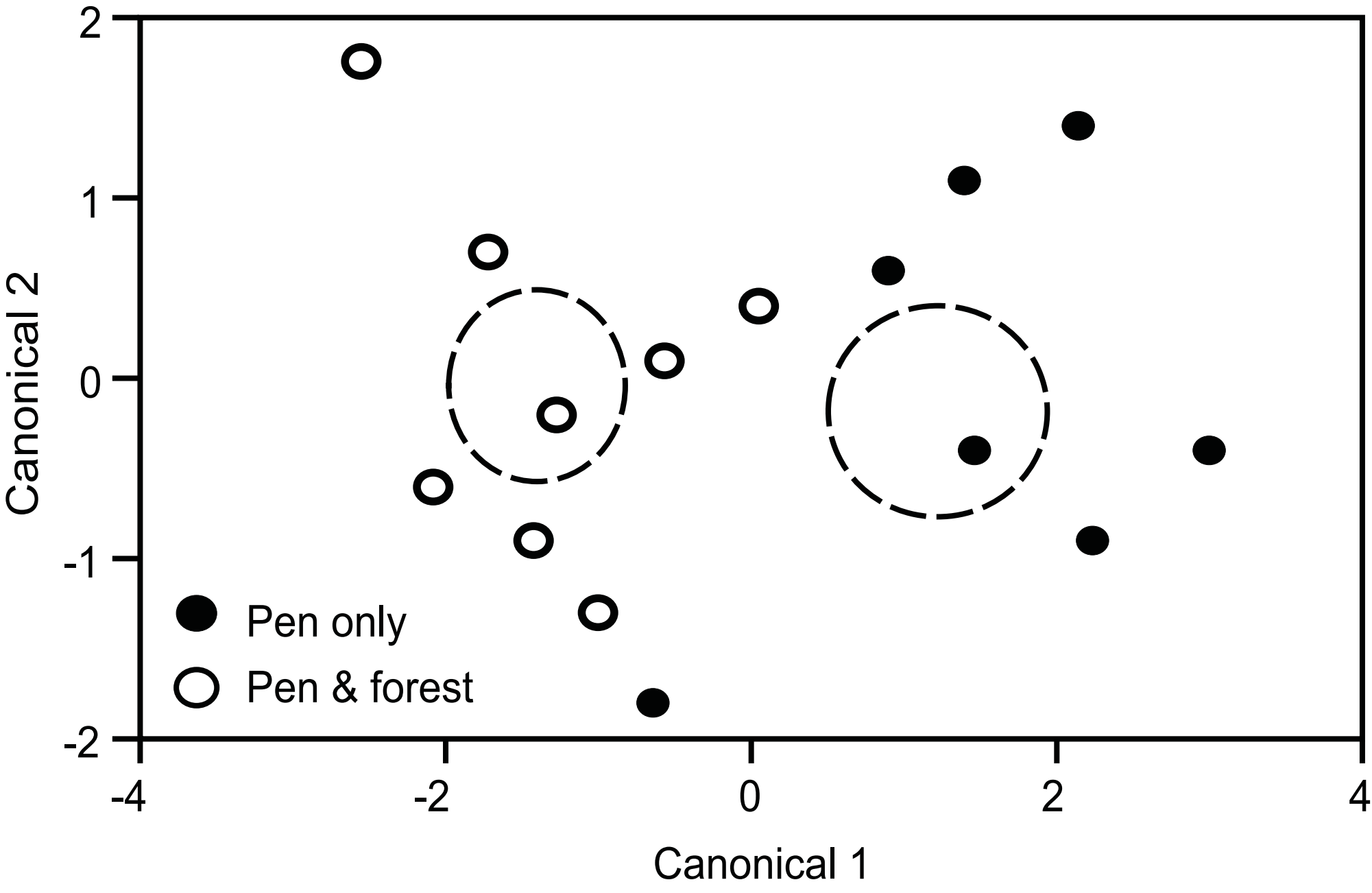
Sifakas expressed more compounds in their urine than either NUM or UM species. The mean (± SD) number of compounds for NUM species was 5.34 ± 2.31, for UM species was

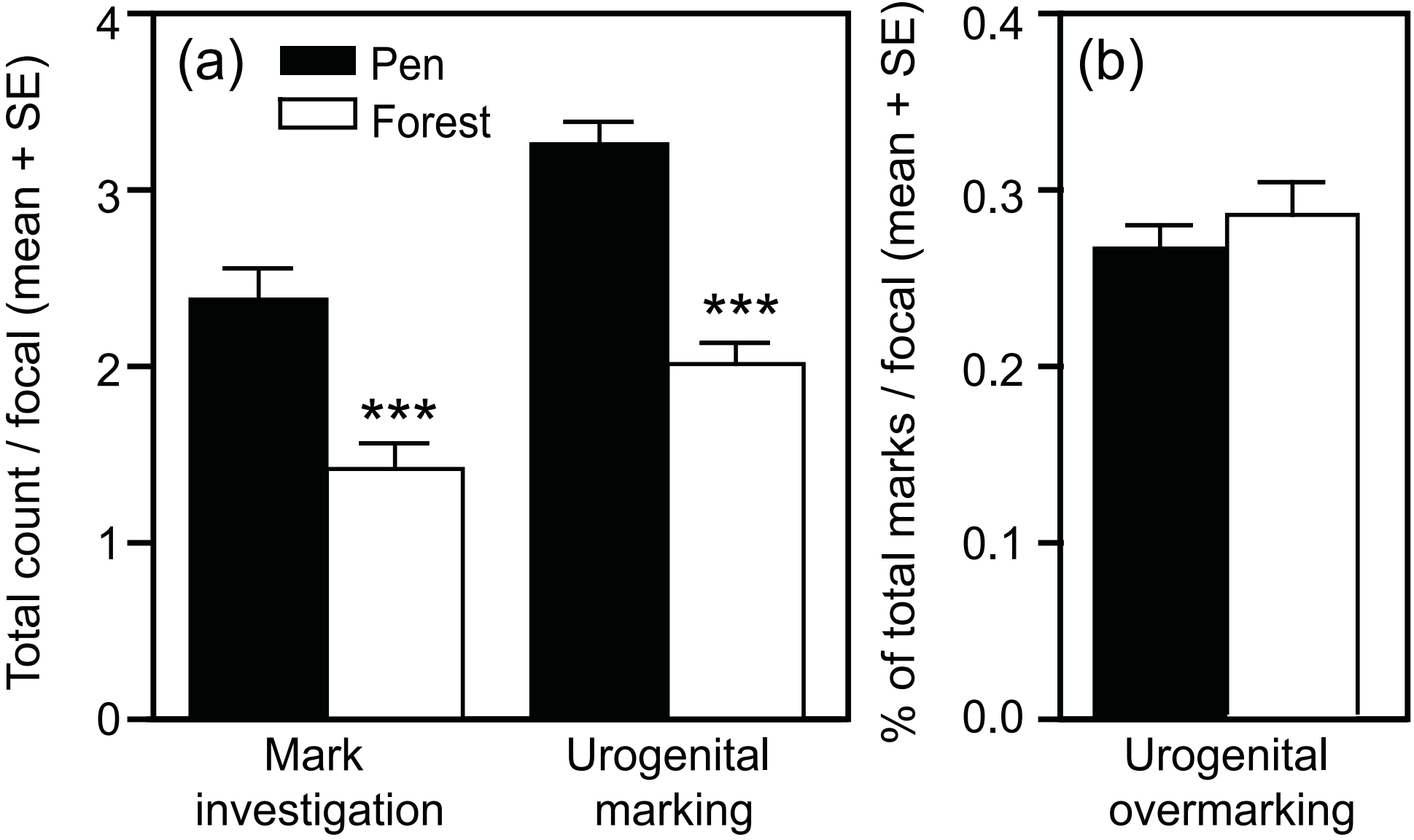
10.06 ± 3.89, and for sifakas was 17.44 ± 9.33. Notably, the mean chemical richness for sifakas was nearly 2 standard deviations greater that the NUM mean, and more than 5 standard deviations greater than the UM mean.

Figure S3. Linear discriminant (LD) plots showing the influence of housing condition on the principal chemical components of sifaka genital secretions. Individuals experiencing the pen condition only (black circles) did not differ from individuals that experienced both the pen and forest conditions (white circles). LD analysis of the 10 principle components retained for this analysis misclassified 6.7 % of samples to the incorrect housing condition and failed to show a statistical difference between groups (Wilks' $\lambda = 0.30$, $P = 0.59$). These findings are in accord with prior analyses in other strepsirrhines, showing a lack of dietary effects on scent chemistry (Drea et al. 2013).

Figure S4. Influence of housing condition on the frequency of olfactory behaviour. At the time of observation, individuals housed in pens (black bars) differed from individuals housed in the forest (white bars) in their frequencies of mark investigation (MI) (GLMM: $Z = 5.17$, $P < 0.0001$) and urogenital marking (UGM) (GLMM: $Z = 6.30$, $P < 0.0001$), but not in the proportion of overmarking (OM) (GLMM: $Z = 0.22$, $P = 0.82$) (***$P < 0.0001$).


(a)
NUM
UM
Sifaka
Chemical richness (mean + SE)
25
20
15
10
5
0
33
23
9
Species
(b)
NUM
UM
Sifaka
Chemical richness (mean + SE)
25
20
15
10
5
0
3
11
4
5
6
4
2
3
6
7
5
9
V.variegata
L.catta
E.flavifrons
E.coronatus
E.mongoz
E.rufus
G.moholi
L.tardigradus
N.pygmaeus
D.madagascariensis
C.medius
P.coquereli
Species


2
1
0
-1
-2
Canonical 2
-4
-2
0
2
4
Canonical 1
Pen only
Pen & forest


(a)
Pen
Forest
Total count / focal (mean + SE)
4
3
2
1
0
***
***
Mark investigation
Urogenital marking
(b)
% of total marks / focal (mean + SE)
0.4
0.3
0.2
0.1
0.0
Urogenital overmarking